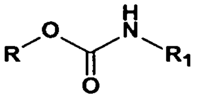
Table 1.

Structures of selected carbamate inhibitors of FAAH activity



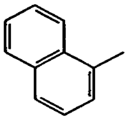
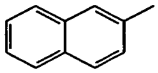
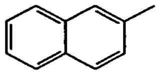
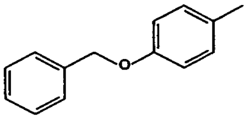
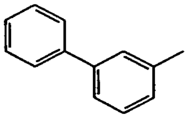
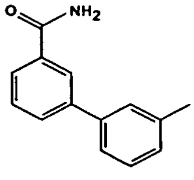
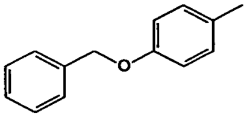
	R	R_1	IC_{50}(nM)	S.E.M.
1		CH_3	>100,000	
2		CH_3	18,600	708
3		c-C_6H_{11}	324	31
4		n-C_4H_9	396	63
5		c-C_6H_{11}	63	9
6		c-C_6H_{11}	4.6	1.6
7		p-$C_6H_{10}F$	>100,000	

Table 2.

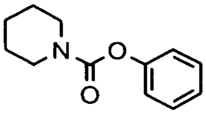
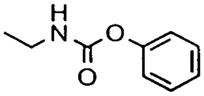
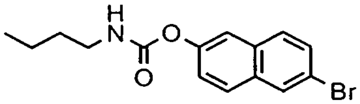
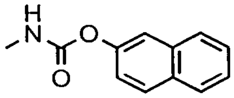
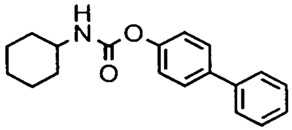
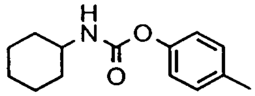
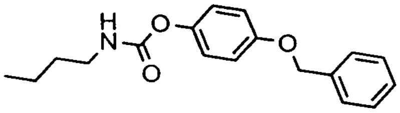
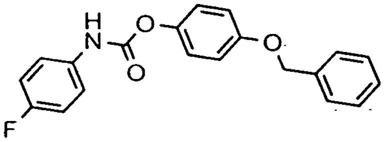
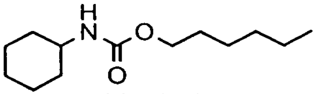
Analysis of selected FAAH inhibitors *in vitro*

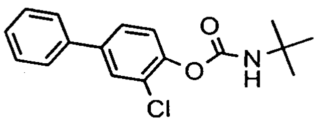
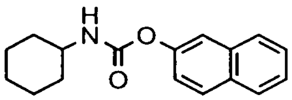
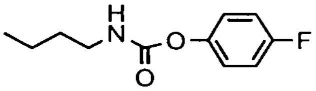
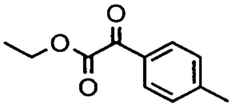
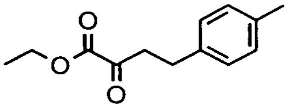
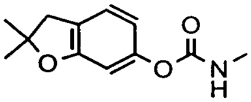
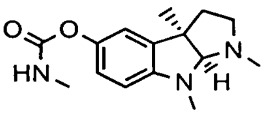
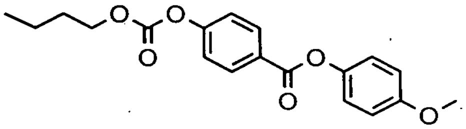
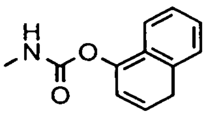
Compound	AChE	SI	BCh	SI	MGL	SI	AT	SI	CB1	SI	CB2	SI
Fenoxbutamate	> 100	≥ 333	> 100	≥ 333	> 30	≥ 100	> 300	≥ 1000	> 300	≥ 1000	> 300	≥ 1000
Carbifenamate	> 100	≥ 25000	≥ 100	≥ 25000	> 30	≥ 7500	> 30	≥ 7500	> 100	≥ 25000	> 100	≥ 25000

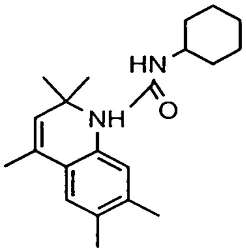
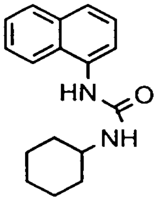
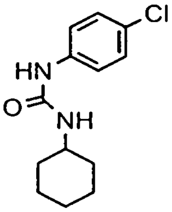
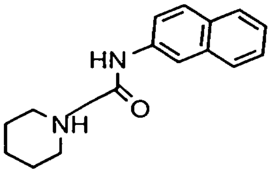
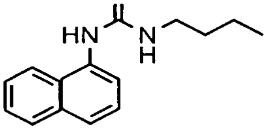
Table 3. Selectivity of various compounds for Inhibition of FAAH and Cholinesterase

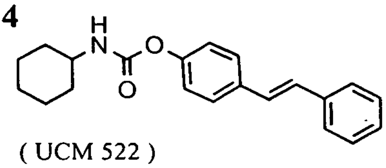
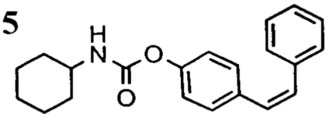
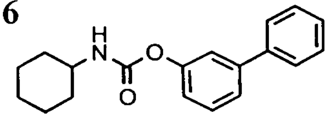
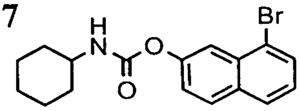
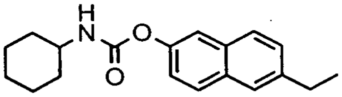
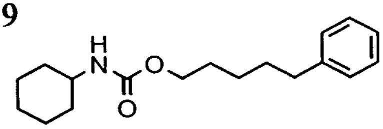
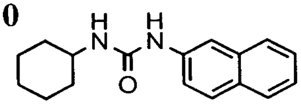
Compound	Name of the compound	Amidohydrolase IC50 (μM)	Cholinesterase IC50 (μM)
PL #1	1-phenyl-N-piperidine carboxylate	> 30	> 30
PL #2	4-tolyl N-ethylcarbamate	> 30	> 30
PL #3	6-bromo-2-napthylyl N-butylcarbamate	0.76	> 30
PL #4	2-napthyl N-methyl carbamate	18.7	> 30
PL #5	4-biphenyl N-cylcohexylcarbamate	2.3	> 30
PL #6	p-tolyl-N-cyclohexylcarbamate	5.4	> 30
PL #7	4-(benzyloxy) phenyl N-butyl carbamate	0.4	> 30
PL #8	4-(benzyloxy) phenyl N-(4-fluorophenyl)carbamate	> 30	> 30
PL #9	hexyl N-cyclohexyl carbamate	3.7	> 30
PL #10	3-chloro-4-biphenyl N-tertbutylcarbamate	> 30	> 30
PL #11	2-napthyl N-cyclohexylcarbamate	0.33	> 30
PL #12	4-fluorophenyl N-butyl carbamate	11.5	> 30
PL #13	ethyl benzylformate	> 30	> 30
PL #14	ethyl-2-oxo-4-phenylbutyrate	25.6	> 30
PL #15	2,3-dihydro-2,2-dimethyl-7-benzofuranol N-methylcarbamate	> 30	0.56
PL #16	physostigmine sulfate	> 30	0.58
PL #17	O-butyl 4-(4'methoxyphenoxycarbonyl)-phenyl carbonate	9.1	> 30
PL #18	carbaryl	> 30	3.1

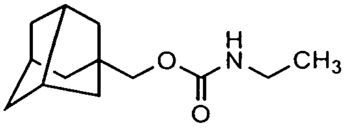
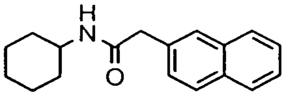
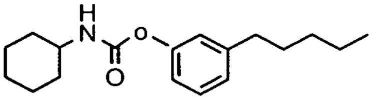
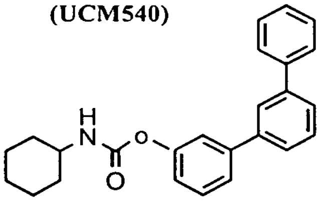
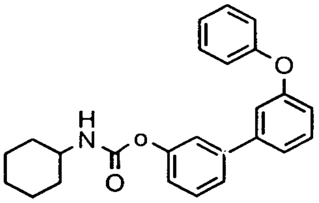
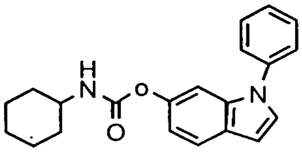
Table 4.

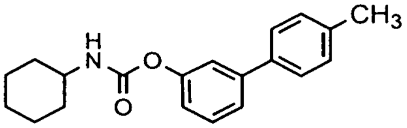
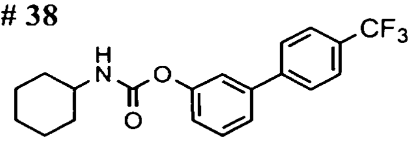
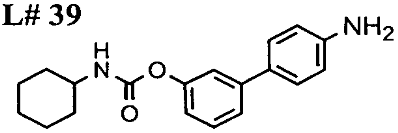
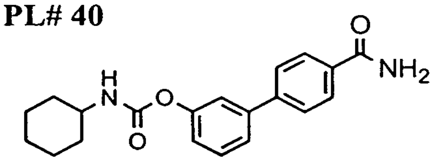
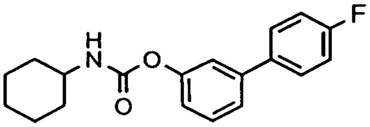
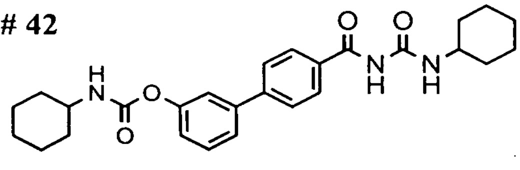
	Inhibition of Amidohydrolase IC_{50} (µM)	Inhibition of Cholinesterase IC_{50} (µM)	CB1 and CB2 Receptor Binding EC_{50} (µM)
PL#1  1-phenyl-N-piperidinecarboxylate	>30		
PL#2  4-tolyl N-ethylcarbamate	>30		
PL#3  6-bromo-2-naphtyhyl N-butylcarbamate	0.76		
PL#4  2-naphtyl-N-methylcarbamate	18.6		
PL#5  4-biphenyl N-cyclohexylcarbamate	2.3		
PL#6  p-tolyl-N-cyclohexyl carbamate	5.4		
PL#7  4-(benzyloxy) phenyl N-butylcarbamate (UCM 532)	0.3		
PL#8  4-(benzyloxy) phenyl N-(4-fluorophenyl)carbamate (UCM 531)	>30		
PL#9  hexyl-N-cyclohexyl carbamate	3.7		

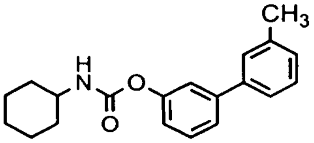
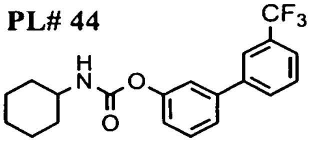
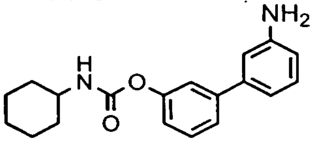
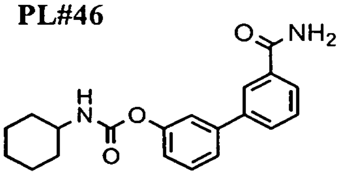
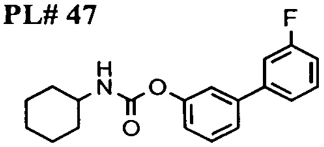
		Inhibition of Amidohydrolase IC$_{50}$ (μM)	Inhibition of Cholinesterase IC$_{50}$ (μM)	CB1 and CB2 Receptor Binding EC$_{50}$ (μM)
PL#10	 3-chloro-4-biphenylyl N-tert-butylcarbamate	>30		
PL#11	 2-naphthyl N-cyclohexylcarbamate	0.33		
PL#12	 4-fluorophenyl N-butylcarbamate	11.5		
PL#13	 ethyl benzylformate	>30		
PL#14	 ethyl-2-oxo-4-phenylbutyrate	25.6		
PL#15	 2,3-dihyrdo-2,2-dimethyl-7-benzofurnaol N-methylcarbamate	>30		
PL#16	 (-)-Physostigmine sulfate	>30		
PL#17	 O-butyl 4-(4'-methoxyphenoxycarbonyl)-phenyl carbonate	9.1		
PL#18	 carbaryl	>30		

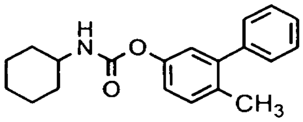
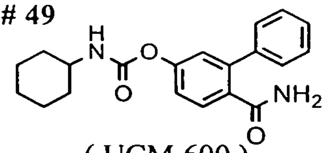
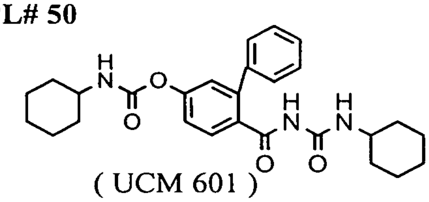
	Inhibition of Amidohydrolase IC_{50} (μM)	**Inhibition of Cholinesterase IC_{50} (μM)**	**CB1 and CB2 Receptor Binding EC_{50} (μM)**
PL#19  CNC-523605	>30		
PL#20  CNC-540335	>30		
PL#21  CNC-541078	>30		
PL#22  CNC-608455	>30		
PL#23  CNC-1072849	>30		

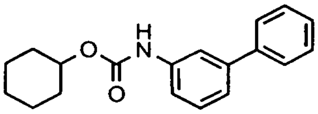
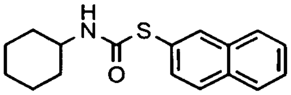
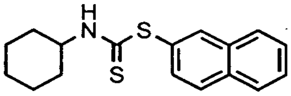
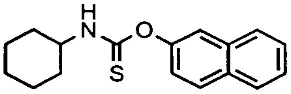
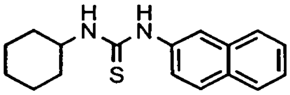
	Inhibition of Amidohydrolase IC$_{50}$ (μM)	Inhibition of Cholinesterase IC$_{50}$ (μM)	CB1 and CB2 Receptor Binding EC$_{50}$ (μM)
PL#24  (UCM 522)	3.3		
PL#25  (UCM 523)	0.24		
PL#26  (UCM 524)	0.06		
PL#27  (UCM 525)	0.17		
PL#28  (UCM 526)	3.0		
PL#29  (UCM 527)	>30		
PL#30  (UCM 528)	>30		

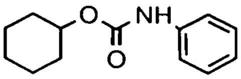
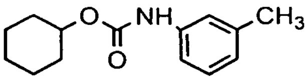
PL compounds		**Inhibition of Amidohydrolase IC_{50} (μM)**	**Inhibition of Cholinesterase IC_{50} (μM)**	**CB1 and CB2 Receptor Binding EC_{50} (μM)**
#31.	 CNC-256597	>30		
#32	 (UCM533)	>30		
#33	 (UCM540)	**0.22**		
#34	 (UCM566)	**0.56**		
#35	 (UCM564)	**2.6**		
#36	 (UCM561)	**1.4**		

	Inhibition of Amidohydrolase IC_{50} (μM)	Inhibition of Cholinesterase IC_{50} (μM)	CB1 and CB2 Receptor Binding EC_{50} (μM)
PL# 37  (UCM 588)	**0.23**		
PL# 38  (UCM 589)	**1.6**		
PL# 39  (UCM 590)	**0.51**		
PL# 40  (UCM 591)	**>10**		
PL#41  (UCM 592)	**0.09**		
PL# 42  (UCM 593)	**~30**		

	Inhibition of Amidohydrolase IC_{50} (μM)	**Inhibition of Cholinesterase IC_{50} (μM)**	**CB1 and CB2 Receptor Binding EC_{50} (μM)**
PL# 43  (UCM 594)	**0.1**		
PL# 44  (UCM 595)	**0.13**		
PL#45  (UCM 596)	**0.06**		
PL#46  (UCM 597)	**0.004**		
PL# 47  (UCM 598)	**0.092**		

	Inhibition of Amidohydrolase IC_{50} (μM)	**Inhibition of Cholinesterase IC_{50} (μM)**	**CB1 and CB2 Receptor Binding EC_{50} (μM)**
PL# 48  (UCM 599)	**0.2**		
PL# 49  (UCM 600)	**0.2**		
PL# 50  (UCM 601)	**3.1**		

	Inhibition of Amidohydrolase IC_{50} (μM)	Inhibition of Cholinesterase IC_{50} (μM)	CB1 and CB2 Receptor Binding EC_{50} (μM)
PL# 51  (UCM 602)	>30		
PL# 52  (UCM 603)	15		
PL# 53  (UCM 604)	>30		
PL# 54  (UCM 605)	>30		
PL# 55  (UCM 606)	>30		

	Inhibition of Amidohydrolase IC_{50} (μM)	**Inhibition of Cholinesterase IC_{50} (μM)**	**CB1 and CB2 Receptor Binding EC_{50} (μM)**
PL#56  (UCM 614)	**3.1**		
PL#57  (UCM 615)	**0.8**		

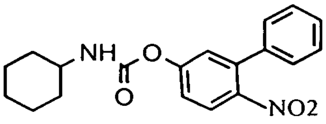
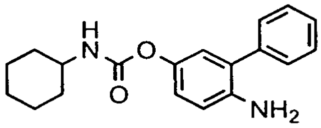
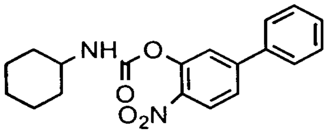
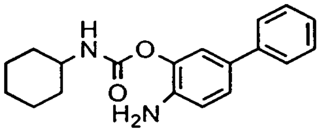
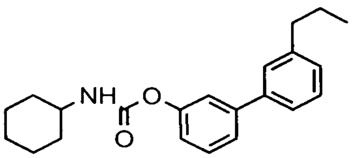
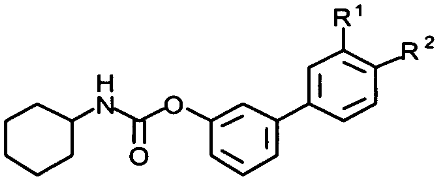
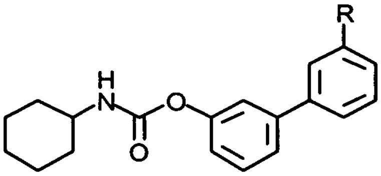
	Inhibition of Amidohydrolase IC_{50} (μM)	Inhibition of Cholinesterase IC_{50} (μM)	CB1 and CB2 Receptor Binding EC_{50} (μM)
PL# 58  (UCM 617)	>30		
PL# 59  (UCM 618)	0.05		
PL# 60  (UCM 619)	>30		
PL# 61  (UCM 620)	>30 ?		
PL# 62  (UCM 621)	0.08		

Table 5. Inhibitory Potencies (IC_{50}) of Tested Compounds on FAAH activity.



Cpds	R^1	R^2	IC_{50} (nM) ± S.E.M.
	H	H	63±9
3a	H	CF_3	1,587±148
3b	H	CH_3	155.4±21
3c	H	$C(O)NH_2$	5,909±951
3d	H	NH_2	360±59
3e	H	F	64.95±14.00
3f	H	$C(O)NHC(O)NH\textit{c}\text{-}C_6H_{11}$	3,017±688
3g	CF_3	H	145.7±16.0
3h	CH_3	H	61.75±19.00
3i	$C(O)NH_2$	H	4.6±1.6
3j	NH_2	H	64.6±9.0
3k	F	H	96.6±4.0
3l	$OC(O)NH\textit{c}\text{-}C_6H_{11}$	H	361±137
3m	C_6H_5O	H	420±86
3n	C_6H_5	H	565±42
3o	$CH_2C_6H_5$	H	1,857±57
3p	$\textit{n}\text{-}C_3H_7$	H	110±16
3q	NO_2	H	49.6±2.0
3r	SO_2NH_2	H	26.5±4.5
3s	$C(O)CH_3$	H	9.1±0.5
3t	CN	H	33.9±7.0
3u	OH	H	8.65±0.10
3v	CH_2OH	H	8.67±0.90
3w	$(CH_2)_2OH$	H	18.7±4.5
3z	CH_2NH_2	H	21,177±7,277

Table 6. Observed and Calculated pIC_{50} Values for FAAH Inhibition of the Meta-substituted Derivatives Included in QSAR Analysis.



Cpds	R	pIC_{50}	
		obsd	calcd
URB524	H	7.20	7.28
3g	CF_3	6.84	6.83
3h	CH_3	7.21	6.99
3i	$C(O)NH_2$	8.34	7.99
3j	NH_2	7.19	7.86
3k	F	7.02	7.19
3l	$OC(O)NH\textit{c}\text{-}C_6H_{11}$	6.44	6.74
3m	C_6H_5O	6.38	6.24
3n	C_6H_5	6.25	6.30
3o	$CH_2C_6H_5$	5.73	6.28
3p	$n\text{-}C_3H_7$	6.96	6.50
3q	NO_2	7.30	7.40
3r	SO_2NH_2	7.58	8.15
3s	$C(O)CH_3$	8.04	7.53
3t	CN	7.47	7.54
3u	OH	8.06	7.59
3v	CH_2OH	8.06	7.76
3w	$(CH_2)_2OH$	7.73	7.64

Figure 1.

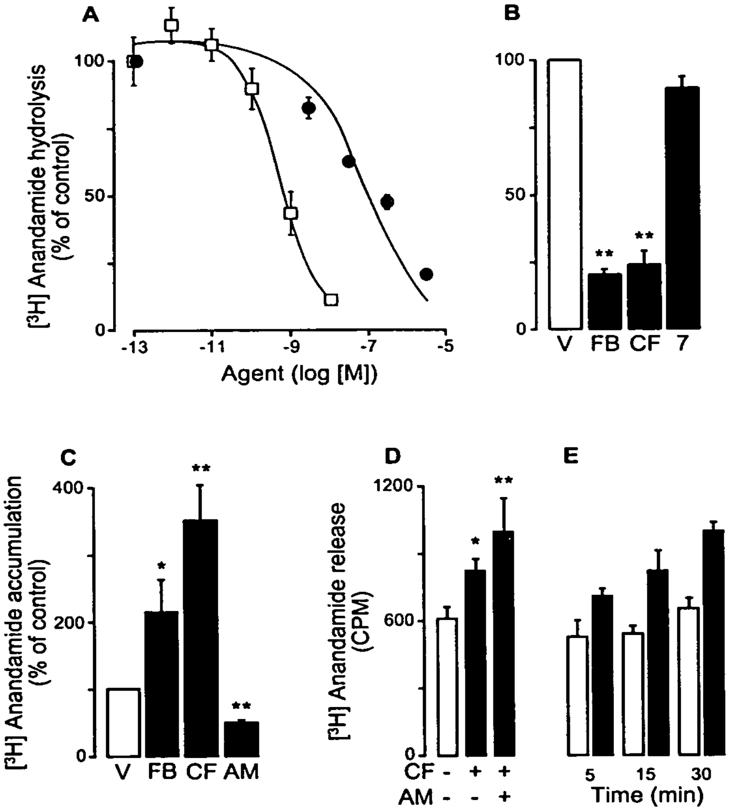

A
B
C
D
E
[³H] Anandamide hydrolysis (% of control)
100
50
0
-13
-11
-9
-7
-5
Agent (log [M])
**
**
V
FB
CF
7
[³H] Anandamide accumulation (% of control)
400
200
0
*
**
**
AM
[³H] Anandamide release (CPM)
1200
600
0
CF - + +
AM - - +
5
15
30
Time (min)

Figure 2.

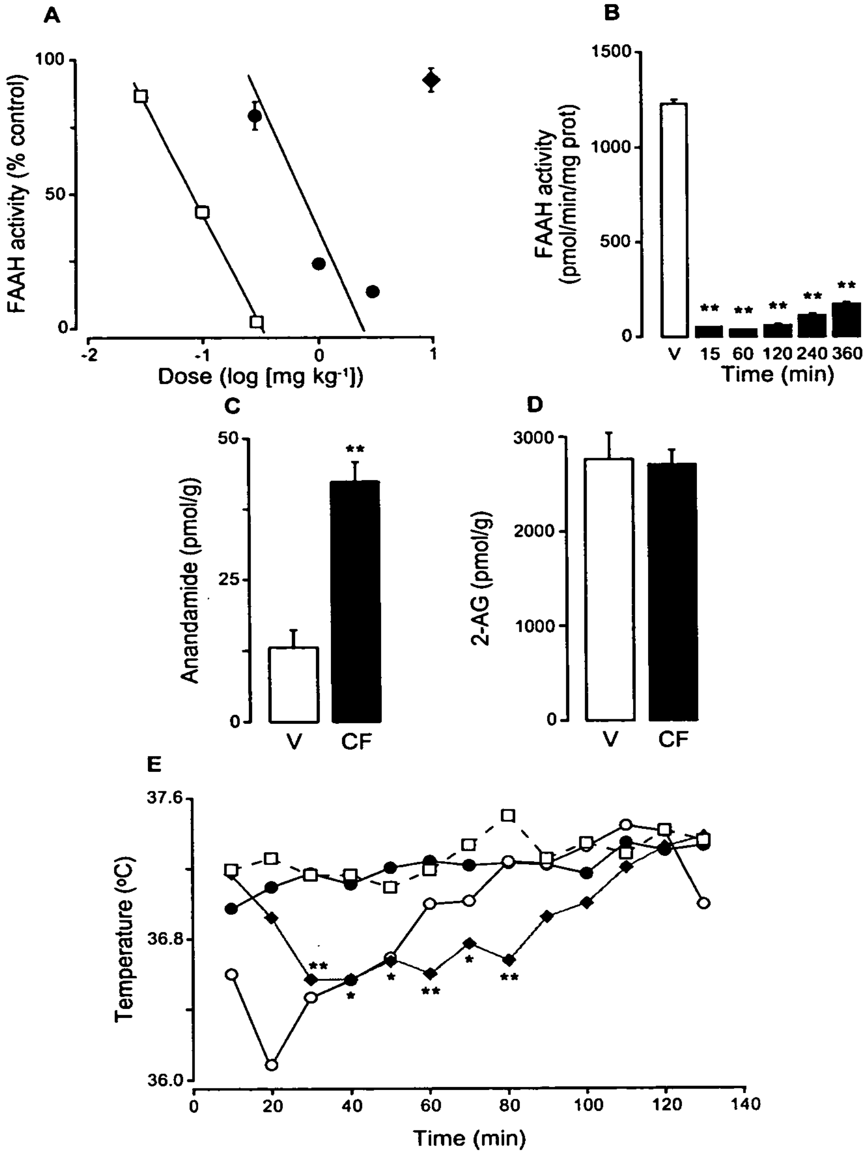

A
FAAH activity (% control)
100
50
0
-2
-1
0
1
Dose (log [mg kg-1])
B
FAAH activity (pmol/min/mg prot)
1500
1000
500
0
**
V
15
60
120
240
360
Time (min)
C
Anandamide (pmol/g)
50
25
0
**
V
CF
D
2-AG (pmol/g)
3000
2000
1000
0
V
CF
E
Temperature (oC)
37.6
36.8
36.0
**
*
**
0
20
40
60
80
100
120
140
Time (min)

Figure 3.

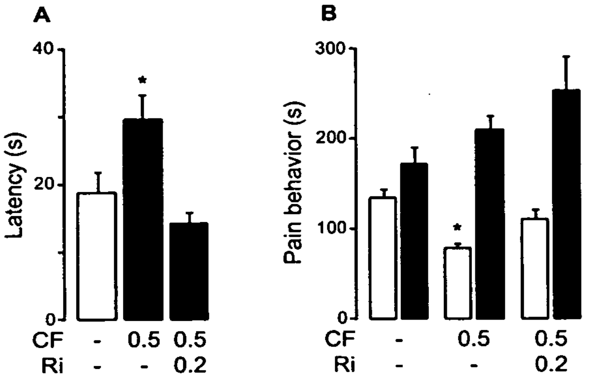

A
40
20
0
Latency (s)
*
CF - 0.5 0.5
Ri - - 0.2
B
300
200
100
0
Pain behavior (s)
*
CF - 0.5 0.5
Ri - - 0.2

Figure 4.

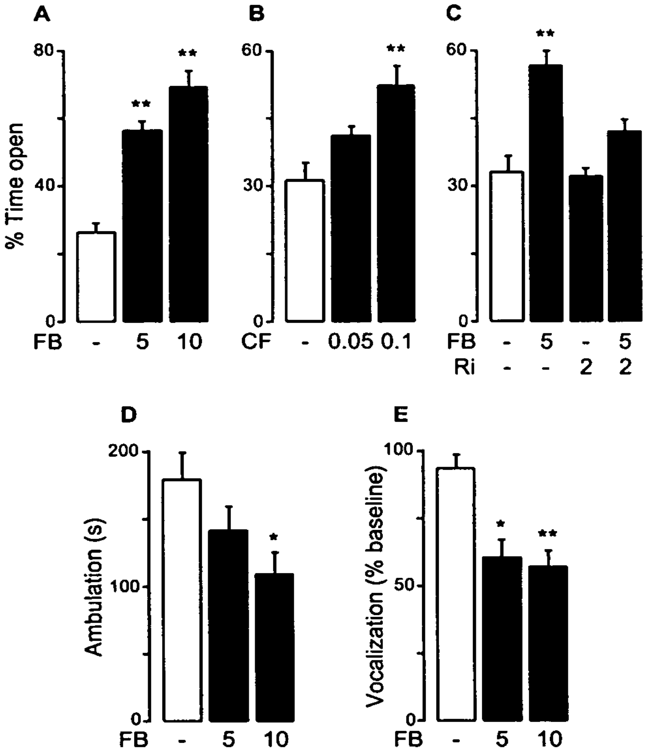
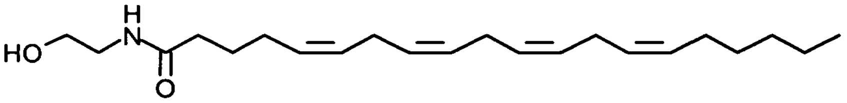
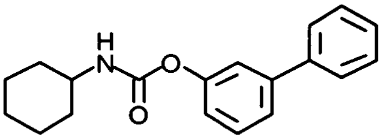

A
B
C
80
40
0
% Time open
**
**
60
30
0
**
60
30
0
**
FB - 5 10
CF - 0.05 0.1
FB - 5 - 5
Ri - - 2 2
D
E
200
100
0
Ambulation (s)
*
100
50
0
Vocalization (% baseline)
*
**
FB - 5 10
FB - 5 10

Figure 5.

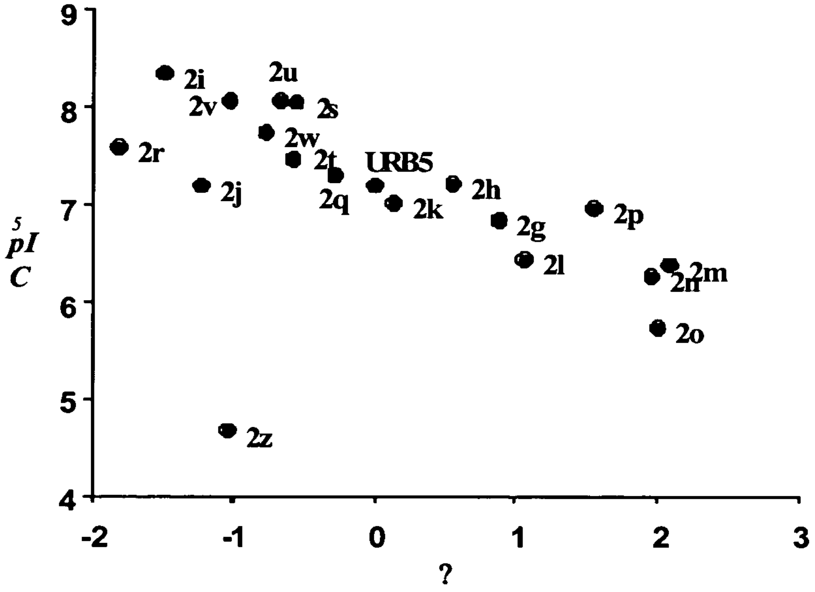


1



2

Figure 6.



9
8
7
6
5
4
5
pI
C
-2
-1
0
1
2
3
?
2i
2u
2v
2s
2r
2w
2t
URB5
2j
2q
2k
2h
2g
2p
2l
2m
2n
2o
2z

Figure 7.

Figure 8.

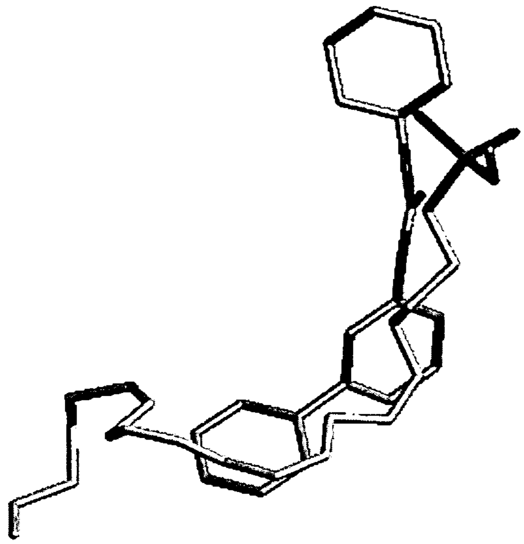


Figure 9.